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Validation of QTL for the Flag Leaf Senescence in Wheat Under Drought

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The study of physiological senescence of the flag leaf playing the pivotal role in the uptake of solar energy and stipulating plant productivity in wheat is very important for providing high productivity under stress. Therefore, using RAPD OPH13 marker, the existence of a gene-locus linked to the physiological flag leaf senescence was examined in wheat genotypes under drought stress. Based on the analysis of PCR profiles, 450 bp diagnostic fragments were identified in 31 bread wheat and in 15 durum wheat genotypes. This result shows that there is a gene locus that provides physiological rejuvenation of the flag leaf, which is an indicator of the resistance to drought stress in those genotypes. The existence of the studied locus was not confirmed in 19% of the genotypes.

Keywords: *Wheat genotypes, flag leaf senescence, drought, PCR, RAPD*

INTRODUCTION

Drought is the crucial environmental stress that has pernicious consequences on agriculture. One of the main goals of breeding programs is to develop varieties exhibiting high tolerance to drought conditions. It is very important to achieve sustainability in agriculture. Despite, fundamental research has provided substantial gains in the understanding of the physiological and molecular responses of plants to drought stress, there is still a large lack between yields in optimal and stress conditions. In cereal crops, the upper three leaves on the stem, especially the top-most leaf, flag leaf are the main source of carbohydrate production (Al-Tahir 2014).

Leaf senescence is a physiological process that causes cell death and is regulated by age under the impact of other endogenous and environmental factors. Leaf senescence is an active and strictly regulated degeneration process. During this process, leaf cells are exposed to strong changes in cell structure, metabolism, and gene expression (Zhao et al., 2018). Leaf senescence generates the change in the leaf cell metabolism. Basically enhanced catabolism leads to a declined assimilation rate e.g., the photosynthetic capacity decreases, and macromolecular material degrades (Lira et. al., 2017). The earliest and most notable modification in the cell structure is the disintegration of the chloroplast, the organelle that contains up to 70% of the leaf protein. Metabolically, carbon assimilation is replaced by catabolism of chlorophyll and macromolecules such as proteins, membrane lipids, and RNA (Lim et al., 2007). Increased catabolic activity is responsible for transforming the cellular materials collected during the growth stage of leaf into exportable nutrients that are directed to developing seeds or to other growing organs. Hence, although leaf senescence is a detrimental process for the whole leaf organ, it can be accepted as a protection process (Senapati et al., 2018). Thus, leaf senescence is an evolutionarily selected developmental process and has a key role in the plant life cycle. According to agricultural reports, leaf senescence may decrease yield in crop plants by reducing the growth phase and may also cause post-harvest spoilage such as leaf yellowing and nutrient loss in vegetable crops.

Programmed cell death (PCD) is a self-destruct cellular process controlled by several factors and mediated through an active genetic program. This process is regulated by numerous active genetic programs. Molecular markers related to quantitative trait loci (QTL) for drought tolerant patterns could increase in breeding for drought conditions. Molecular markers can be used to research germplasm through segregation and association mapping to detect useful alleles in both cultivated and wild relatives. Moreover, association mapping is naturally more powerful than ‘classical’ genetic linkage mapping because it analyzes the results of generations of recombination and selection, most of the data available currently on water deficit are based on segregation mapping and QTL analysis. An advantage of the RAPD technique is that it gives rapid outcome but at the same time has limitations such as low reproducibility (Fernandez et al., 2002).

The main purpose of this research is the identification of QTL for the flag leaf senescence in wheat by RAPD marker.

MATERIALS AND METHODS

Plant materials: Wheat genotypes (38 genotypes

of bread (*Triticum aestivum* L.) and 19 genotypes of durum (*Triticum durum* Desf.) wheat genotypes collected in the Gene Pool of the Research Institute of Crop Husbandry (Baku) acted as a research object. Plants were cultivated in field conditions.

Extraction of plant DNA**:** DNA extraction was carried out using the CTAB method with some modifications (Murray and Thompson, 1980). Fresh plant tissue as a fragment of leaf was minced in liquid nitrogen, suspended in 1000 μl of CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 40 mM β-mercaptoethanol), and pre-warmed in a water bath at 60ºC. Homogenization was completed by intense Vortex shaking. Then 400 ml of chloroform (99.8%) was added into each tube and the tubes were gently mixed. Next the tubes were placed in a water bath and incubated for 10 min at 60 ^{0}C. After incubation, the tubes were centrifuged in an Eppendorf type benchtop centrifuge (15,000 g) for 10 min at room temperature. After centrifugation the supernatant was carefully selected (taking care not to capture sediment particles) and transferred to clean 1.5 ml Eppendorf type tubes and 600 ml of cold isopropanol was added, mixed well and left at room temperature for 3-5 minutes. At this stage we can observe the dispersed DNA precipitate. The tube contents were centrifuged at room temperature in the Eppendorf type benchtop centrifuge (15,000 g) for 10 min.

The precipitate was washed several times with 70% ethanol, dried in a thermostat at 56 ^{0}C for 5 minutes and dissolved in TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA). Samples were left in a refrigerator at 4^{0}C for the complete dissolution of DNA in a buffer.

DNA quantification**:** After dissolution of DNA the quantity was determined by optical density (OD) at λ=260 using the ULTROSPEC 3300 PRO spectrophotometer ("AMERSHAM", USA).

Purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. DNA quality was checked on the basis of performance of the extracted DNA samples on 0.8% agarose gel stained with 10 mg / ml of ethidium bromide in 1 × TBE (Tris base, Boric acid, EDTA) buffer. The gel was developed and photographed under ultraviolet light using "Gel Documentation System UVITEK" (UK).

DNA amplification**:** Polymerase chain reaction was performed by Williams (1990). DNA amplification was performed in a 25 μl reaction mixture volume, containing 10 × buffer, 20 ng of the genomic DNA, 0.2 μM primer, 200 μM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, and 0.2 units of Taq-polymerase in the incubation buffer. PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" (Singapore) thermocycler under the following conditions: 1 cycle - 3 minutes at 94ºC; 38 cycles - 1 min at 94 ºC, an annealing step at variable annealing temperatures depending on the primer pairs for 1 min, 2 minutes at 72 ºC; the final elongation cycle was performed at 72 °C for 10 min, then kept at 4°C.

The reaction products were separated by electrophoresis on a 3% agarose gel in the HR-2025-High Resolution («IBI SCIENTIFIC» U.S.) horizontal electrophoresis machine with addition of ethidium bromide and documented using «Gel Documentation System UVITEK». Sizes of amplified fragments were determined with respect to 100 bp DNA marker. Statistical analysis included binary matrix compilation for each of the primers, in which "presence" (1) or "absence" (0) of fragments with equal molecular weight on the electropherogram were noted.

RESULTS AND DISCUSSION

Drought stress is the main factor affecting grain yield and leaf senescence in wheat. Leaf senescence causes significant changes at the cellular, tissue, organ, and organism levels. In this work QTL for flag leaf senescence has been researched under drought stress using RAPD marker. 57 wheat genotypes collected in the Gene Pool of the Research Institute of Crop Husbandry acted as research objects. 38 of them were bread wheat, and 19 were durum wheat genotypes. Plants were cultivated under field conditions. RAPD marker OPH13 (5`GACGCCACAC3`) linked to the QTL for flag leaf senescence (Milad et al. 2011) was used for the screening

As can be seen in Table 3, RAPD OPH13 gives a positive result in 46 genotypes, this is approximately 81% of all genotypes used for this analysis. In more detail, 450 bp diagnostic fragments were identified in 31 bread wheat and in 15 durum wheat genotypes. This result shows that there is a gene locus that provides physiological rejuvenation of the flag leaf, which is an indicator of the resistance to drought stress in those genotypes. Amplification products were absent in 11 genotypes: 7 samples among them were bread wheat, the remaining 4 were durum wheat. The Primer OPH13 (5` GACGCCACAC 3') produced a strong polymorphic band at 450 bp (Fig. 1).

Wheat flag leaf has a key role during photosynthesis in absorption solar energy and therefore, flag leaf senescence is one of the main parameters to provide high productivity. Leaf senescence is induced not only by hormonal factors due to plant aging, external environmental factors, such as high temperature and drought can also be the reason of premature senescence (Chandler 2001).

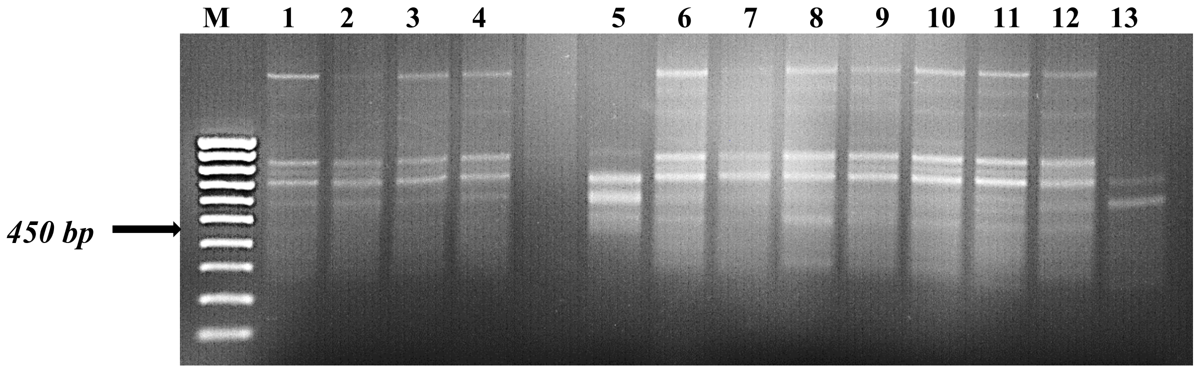


Fig. 1. PCR-profiles of wheat genotypes for RAPD OPH13. M - 100 bp DNA ladder, 1- Gilavar, 2- Sevinj, 3- Gyzyl bughda, 4- Garabagh, 5- Garagylchyg 2, 6- Yagut, 7- Shirvan 3, 8- Tartar, 9- Parvin, 10- Saba, 11- Ugur, 12- Aran, 13- Shiraslan 23, Arrow shows ~ 450 bp band.

Table 3. Results of PCR analysis using RAPD markers OPH13. [+] – presence of the expected locus, [-] – absence of this locus.

T.aesivum **L.**			
Genotypes	OPH13 (450bp)	Genotypes	OPH13 (450 bp)
Parvin	+	Dagdash	+
Gilavar	-	Giymatli 2/17	+
Saba	+	Farandole	+
Layaqatli 80	+	Saratovskaya 29	+
Bayaz	+	Tale 38	+
Mahmud 80	+	A2	+
Murov 2	+	Miranovka	+
Pirshahin	+	Ruzi 84	+
Aran	+	1st WWEERYT	+
Ugur	+	Mirbashir 128	+
Zirva 85	+	Renan	+
Nurlu 99	+	11thFAWWON №	-
№97 12th FAWWON	-	Gyrmyzy gul 1	+
№50 4th FEFWSN	-	Akinchi 84	+
Parzivan	-	Azamatli 95	+
Fatima	+	Murov	+
Shaki 1	+	Sevinj	-
Farahim 2012	+	Gyzyl bugda	-
Qualite	+	Fransa	+
T.durum **Desf.**			
Shirvan 3	-	Tigre	+
Mirvari	+	Gyrmyzy bugda	+
Turan	+	Garabagh	-
Vugar	+	Sharg	+
Tartar	+	Ag bugda	+
Yagut	+	Kakhraba	+
Mirbashir 50	+	Garagylchyg 2	+
Mugan	+	Shiraslan 23	-
Asgaran	+	Sarychanak 98	+
Barakatli 95	-		

. Biochemical and physiological events lead to leaf senescence, which is the final stage of leaf development. In wheat flag leaf senescence occurs when redistributing resources from the source to the sink during grain filling. The onset and rate of senescence are main determinants of yield potential (Evans 1993), because flag leaf photosynthesis in wheat contributes about 30–50% of the assimilates for grain filling (Sylvester-Bradley et al. 1990). Four classes of late senescence or ‘stay-green’ were described by Thomas and Smart (1993). Two of these classes relate to delayed onset of senescence or slower rate in progress of senescence, and the remaining two relate to cosmetic effects that lack photosynthetic capability. There were some reports on the inheritance of flag leaf senescence in wheat

under optimal conditions, where additive gene effects were demonstrated (Simon 1999). It was found that delayed onset of leaf senescence in sorghum (*Sorghum bicolour* L.) (Borrell et al. 2000a, 2000b), maize (*Zea mays* L.) (Baenziger et al. 1999) and durum wheat (*T. durum* L.) (Benbella and Paulsen 1998; Hafsi et al. 2000) increased plant productivity under water stress conditions. In sorghum a slower rate of senescence was also associated with increases in genetic yield under drought (Borrell et al. 2000a, 2000b).

It is necessary for plant breeders to understand the genetics of leaf senescence for increasing yield under drought. Moreover, this would allow the scientist to elucidate how genes and biochemical pathways controlling leaf senescence are regulated.

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Buğda Bitkisində Quraqlıq Stresi Şəraitində Flaq Yarpağın Qocalması ilə Əlaqədar QTL-in Tədqiqi

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Buğda bitkisində fotosintez zamanı günəş enerjisinin udulmasında əsas rol oynayan və bitkinin məhsuldarlığını şərtləndirən flaq yarpağın fizioloji qocalmasının tədqiq olunması stress şəraitində yüksək məhsuldarlığın təmin edilməsi üçün vacib parametrlərdən biridir. Bu baxımdan, RAPD OPH13 markerindən istifadə etməklə buğda genotiplərində stress şəraitində flaq yarpağın fizioloji qocalması ilə əlaqəli gen-lokusunun mövcudluğu tədqiq edilmişdir. PZR nəticələrindən əldə olunan elektroforetik profillərin analizinə əsasən, 31 yumşaq və 15 bərk buğda genotipində 450 bp diaqnostik fraqmentlər aşkar edilmişdir. Bu nəticə onu göstərir ki, genotiplərdə quraqlıq stresinə qarşı davamlılığın göstəricisi hesab olunan flag yarpağın fizioloji yaşılqalmasını təmin edən gen lokusu mövcuddur. Araşdırılan gen lokusu genotiplərin 19 %-də aşkar edilməmişdir.

Açar sözlər: *Buğda genotipləri, flag yarpağın qocalması, quraqlıq, PZR, RAPD*

Исследование Связанного со Старением Флангового Листа QTL у Пшеницы при Условиях Засухи

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Изучение физиологического старения флаговых листьв, играющих ключевую роль в поглощении солнечной энергии и определении продуктивности растений пшеницы, очень важно для обеспечения высокой производительности при стрессе. В генотипах пшеницы при условиях засухи, с помощью маркера RAPD OPH13, было исследовано наличие ген-локуса, связанного с процессом старения флаговых листьев. Основываясь на анализе ПЦР, в 31 генотипе мягкой и 15 – генотипах твердой пшеницы, были идентифицированы диагностические фрагменты 450 bp. Этот результат указывает на наличие локуса, обеспечивающего физиологическое озеленение флагового листа, который является критерием устойчивости к стрессу засухи у этих генотипов. Изученный локус не был обнаружен у 19% генотипов.

Ключевые слова: *Генотипы пшеницы, старение флагового листа, засуха, ПЦР, RAPD*